Invited Review

Transcranial Cerebellar Direct Current Stimulation and Transcutaneous Spinal Cord Direct Current Stimulation as Innovative Tools for Neuroscientists

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Abstract

Two neuromodulatory techniques based on applying direct current (DC) non-invasively through the skin, transcranial cerebellar direct current stimulation (tDCS) and transcutaneous spinal DCS, can now induce prolonged functional changes consistent with a direct influence on the human cerebellum and spinal cord. In this article we review the major experimental works on cerebellar tDCS and on spinal tDCS, and their preliminary clinical applications. Cerebellar tDCS modulates cerebellar motor cortical inhibition, gait adaptation, motor behaviour, and cognition (learning, language, memory, attention). Spinal tDCS influences the ascending and descending spinal pathways, and spinal reflex excitability. In the anaesthetised mouse, DC stimulation applied under the skin along the entire spinal cord may affect GABAergic and glutamatergic systems. Preliminary clinical studies in patients with cerebellar disorders, in animals and patients with spinal cord injuries have reported beneficial effects. Overall the available data show that cerebellar tDCS and spinal tDCS are two novel approaches for inducing prolonged functional changes and neuroplasticity in the human cerebellum and spinal cord, and both are new tools for experimental and clinical neuroscientists.

Introduction

Two novel and simple methodological approaches described by our group and developed in the past six years now suggest that direct current (DC) delivered transcutaneously can modulate functions in the cerebellum (Block & Celnik, 2012; Ferrucci & Priori, 2013; Grimaldi *et al.*, 2013; Tomlinson *et al.*, 2013) and spinal cord (Cogiamanian *et al.*, 2012; Lamy & Boakye, 2013) over a prolonged time. The idea of using transcutaneous DC for modulating function in the human cerebellum and spinal cord arose from observation that DC delivered through the scalp modulate brain excitability (Priori *et al.*, 1998; Nitsche & Paulus, 2000; Priori, 2003; Ardolino *et al.*, 2005; Brunoni *et al.*, 2012) and hence DC could also modulate other central nervous system (CNS) structures. As happens for brain tDCS, novel approaches using DC applied transcutaneously presumably act at least in part by polarising the neuronal membrane and inducing neuroplasticity.

The human cerebellum has unique functions, correlated with motor control, learning, cognition, emotions and behaviours (Strick *et al.*, 2009; Manto & Haines, 2012; Reeber *et al.*, 2013). Though the cerebellum accounts for only approximately 10% of the brain volume, it contains more than half of all neurons. Such a high neuronal concentration may in part account for cerebellar sensitivity to the electric field. The various cerebellar functions arise from its division into three separate portions each having different connections with the rest of the CNS: the vestibulocerebellum (with efferent and afferent connections to the vestibular nuclei), the spinocerebellum (with afferent connections from the spinal cord and efferent connections controlling the medial and lateral descending motor systems), and the cerebrocerebellum (efferent and afferent connections with the cerebral cortex (Ghez & Thac, 2000). Because no drugs can yet primarily influence cerebellar dysfunction in pathological conditions, cerebellar stimulation offers a promising therapeutic opportunity. Already in their classic experiments conducted in cats in the 1950s Moruzzi and co-workers recognised cerebellar sensitivity to polarising DC (Mollica *et al.*, 1953d, b, c, a; Gauthier *et al.*, 1955;

Pompeiano & Cotti, 1959a, b). This sensitivity might well underlie the DC-induced changes in human cerebellar functions that we will review here. Because the cerebellum intervenes in several brain activities, cerebellar stimulation might help improve deficits arising from brain lesions. The cerebellum could therefore be a unique "window" through which cerebellar tDCS could modulate functions residing elsewhere in the brain.

Another fascinating target for novel neuromodulatory approaches is the spinal cord. The spinal cord, besides containing the reflex centres, contains sensory, motor and associative propriospinal pathways. Increasing evidence implies that because the spinal cord stores some functions that the brain controls under normal conditions, these could be rescued if the cord is diseased or injured and detached from the rest of the CNS (Hubli *et al.*, 2013). Hence spinal neuromodulation could enhance this rescue and facilitate functional recovery in patients. An innovative view comes also from envisaging the spinal cord as a "highway" to the brain: stimulating or modulating the spinal cord might induce diffuse functional changes other than purely motor or sensory functions, elsewhere in the CNS, such as for example synchronizing the activity in different cortical areas. That the spinal cord, like the cerebellum, is sensitive to polarising DC is again hardly surprising given that research conducted more than 50 years ago showed that even low-intensity DC, <0.5-1mA, can modulate spinal cord function in the cat (Eccles *et al.*, 1962). Although whether results obtained in animals can be translated one-to-one to humans both for cerebellar and spinal tDCS remains unclear owing to various factors (different stimulation intensity and duration, electrode positions and size, and comparative anatomy), these experiments provide a background for the methodologies reviewed here.

Transcranial Cerebellar DC Stimulation (cerebellar tDCS)

The possibility of non invasively stimulating the human cerebellum is not new. Already in 1995, Ugawa first described a technique for delivering transcranial magnetic stimulation (TMS) to the cerebellum with single magnetic pulses. He found that a single TMS shock over

the cerebellum inhibited the motor response evoked by TMS delivered a few ms later over the contralateral motor cortex; Ugawa and co-workers (1995) called this phenomenon cerebello-brain inhibition (CBI). A few years later, experiments with repetitive transcranial magnetic stimulation (rTMS) over the cerebellum showed that cerebellar rTMS could induce after-effects on cerebellar functions (Theoret *et al.*, 2001). Nonetheless cerebellar tDCS has some practical advantages on rTMS (discussed below). In this section we will describe cerebellar tDCS designs used so far. For cerebellar DC stimulation (anodal or cathodal) an electrode (measuring about 5 x 5 cm) is placed over the cerebellum and the reference electrode over the right arm (Ferrucci *et al.*, 2008; Ferrucci *et al.*, 2012; Pope & Miall, 2012; Ferrucci *et al.*, 2013), or the buccinator muscle (Galea *et al.*, 2009; Boehringer *et al.*, 2012; Galea *et al.*, 2012; Hamada *et al.*, 2012; Jayaram *et al.*, 2012; Sadnicka *et al.*, 2013; Shah *et al.*, 2013; Hardwick & Celnik, 2014; Herzfeld *et al.*, 2014; Zuchowski *et al.*, 2014), or contralateral supra-orbital area (Grimaldi & Manto, 2013; Grimaldi *et al.*, 2014), or the motor cortex (Macher *et al.*, 2014). Although no studies have systematically investigated whether placing the reference electrode on the face or arm when applying cerebellar tDCS influences its effects, electrode positioning is probably an important experimental variable. Both electrodes are connected to a stimulator delivering DC for 15-25 minutes, at an intensity ranging from 1 to 2 mA (Table 1). Cerebellar DC stimulation occasionally elicits short-lasting tingling sensations when stimulation begins and ends and sometimes redness under the electrode.

Motor Control and Neuroplasticity

The physiological rationale for using cerebellar tDCS to influence motor control depends, in brief, on the observation that Purkinje cell activation inhibits the deep cerebellar nucleus neurons ensuring that they receive the correct amount of inhibition to produce the appropriate motor output and suppress unwanted activity. After research in our laboratory described the technique (Ferrucci *et al.*, 2008), the well-known cerebellar involvement in motor control prompted Galea and co-workers (2009) to study how cerebellar tDCS affects

CBI in healthy individuals. CBI is the TMS-elicited motor evoked potentials (MEP) inhibition produced by a single TMS shock over the cerebellum (Ugawa *et al.*, 1995). The main experiment disclosed that cathodal cerebellar tDCS decreased CBI, whereas anodal cerebellar tDCS increased it, and sham stimulation induced no changes. These effects specifically involved the cerebello–cortical connections and no changes were found in other M1 or brainstem excitability variables. These results suggest that cerebellar tDCS can modulate cerebellar control over the brain. Despite the small study sample (individual experiments were conducted in 6-8 subjects, without balancing groups for gender) and the lack of stimulation on a control area (left cerebellum or M1), this is an important study because it reports the first neurophysiological evidence showing the effects of cerebellar tDCS on cerebellar inhibitory output to the motor cortex in healthy subjects.

The functional cerebellum-cerebrum interaction is determinant to plasticity in the somatosensory and motor cortex (Rahmati *et al.*, 2014) and probably also to plasticity in other cortical areas. Cerebellum-dependent brain plasticity is probably important also for complex cognitive tasks with a major timing component. Given that the cerebellum intervenes in neuroplasticity, cerebellar tDCS could induce some effects through neuroplasticity changes and long-term potentiation (LTP). In addition to rTMS and tDCS, another method used to induce neuroplasticity is paired associative stimulation (PAS): this technique entails delivering about two hundred repetitive electrical stimuli over the median nerve at the wrist paired with TMS pulses over the spot of the median-nerve innervated small hand muscles at a rate of about 0.2 Hz. The interstimulus interval between the peripheral nerve shock and TMS ranges between 21.5 and 25 ms. After the PAS protocol ends the median nerve TMS-elicited MEP increases in size (Stefan *et al.*, 2000). The increased motor cortex excitability is thought to arise from LTP-like neuroplasticity changes in human motor cortex (Classen *et al.*, 2004). Assessing whether the cerebellum can influence neuroplasticity changes in the motor cortex, Hamada and co-workers (2012) found that concurrent anodal or cathodal cerebellar tDCS blocked PAS-induced plasticity. They

showed that cerebellar tDCS blocks the PAS-induced LTP-like effects specifically only when the interval elapsing between peripheral and motor cortical stimuli is 25 ms, but not when it is 21.5 ms. They therefore speculated that separate mechanisms mediate PAS-induced changes at these two interstimulus intervals and that PAS25-induced changes depend specifically upon the cerebellum. Although delivering stimuli to a cutaneous control area would have ruled out possible subthreshold influences from sensory input on PAS, the study is overall well-designed. Besides, the conclusion about the different physiological mechanisms underlying PAS bears the important implication that cerebellar stimulation can -- at least in part -- influence normal LTP-like neuroplasticity in the motor cortex. This effect could be useful in pathological conditions such as dystonia, thought to involve abnormal plasticity.

Surround inhibition (SI) is a neurophysiological mechanism by which the CNS focuses neuronal activation (Beck & Hallett, 2011). SI can be studied in the human motor system by observing thenar muscle TMS-elicited MEP inhibition during a voluntary hypothenar muscle contraction and vice-versa (Sohn & Hallett, 2004). Motor SI seems to be based on a GABA-mediated inhibitory mechanism that permits skilled and individuated finger movements. The inhibition appears before the movement execution and reaches its maximum at movement onset (Beck & Hallett, 2010). The cerebellar role in SI is unclear. To assess this issue Sadnicka and co-workers (2013) used cerebellar tDCS to examine the cerebellar role in mSI, but found no evidence that the cerebellum contributes to the neuroanatomical network needed for generating mSI. Although the interval elapsing after cerebellar tDCS ends and the mSI protocol begins is unclear but might have been important for interpreting the results, these findings suggest that the cerebellum has no role in the SI mechanism. The useful finding in this negative study is that it shows that the effects of cerebellar stimulation are specific only for certain physiological mechanisms but not for others.

The cerebellum plays a crucial role in locomotion probably by generating patterned limb

movements, dynamically controlling posture and balance and adjusting the locomotor output by error feed-back learning (Morton & Bastian, 2007; Manto & Haines, 2012). According to this rationale, using cerebellar DC to influence these two functions seemed intriguing. To investigate this possibility, Jayaram and co-workers (2012) used a cerebellum-dependent split-belt walking task to investigate how cerebellar tDCS influences locomotor learning that is controlled by the cerebellum (Morton & Bastian, 2007). In this procedure one leg is set to move faster than the other so that the fast and slow leg steps are asymmetric. Over time, subjects learn to predict and account for the perturbation (Reisman *et al.*, 2005). The investigators studied the laterality in adaptive changes by separately delivering cerebellar tDCS (anodal, cathodal and sham) over the cerebellar hemisphere ipsilateral to fast and slow lower limbs during locomotor adaptation. Anodal cerebellar tDCS applied during walking improved locomotor adaptation, whereas cathodal cerebellar tDCS worsened it but did so only ipsilaterally to the fast leg. Hence the effect was side specific. Even though they provide no information on how long the effect lasts, the results demonstrated that cerebellar tDCS modulates locomotor learning in healthy subjects. This study is important because it provides the first behavioural evidence that cerebellar DC stimulation influences a motor learning task in normal subjects and it opens the avenue to studies in patients with gait abnormalities.

Classic blink reflex conditioning is a simple motor learning form requiring cerebellar integrity. In their study in healthy subjects, Zuchowski, Timmann, and Gerwig (2014) tested the effects of cerebellar tDCS on conditioned eye-blink response acquisition by using a standard delay conditioning paradigm. After anodal tDCS, conditioning was significantly enhanced and after cathodal tDCS was significantly reduced compared with sham stimulation. The authors therefore concluded that eye-blink response conditioning is polarity-dependent and modulated by cerebellar tDCS. This is an important observation demonstrating that simple motor learning forms depend closely on the human cerebellum and can be bidirectionally (increased or decreased) modulated by cerebellar tDCS.

Another brain function that comes under cerebellar control is visuomotor coordination (Brown *et al.*, 1993). The visuomotor cerebellum comprises the floccular lobe, the paraflocculus, the oculomotor vermis, the uvula-nodulus and the ansiform lobule (Voogd *et al.*, 2012). Prompted by this scientific rationale, in a study designed to address the respective cerebellar and M1 roles during adaptive learning, Galea and co-workers (2012) applied anodal cerebellar and motor cortical tDCS during a visuomotor adaptation paradigm. The results showed that the error reduction during adaptation was larger after cerebellar tDCS than after M1 tDCS. In contrast, after tDCS over M1, error adaptation was unchanged but the newly learnt visuomotor transformation (an unexpectedly imposed 30-degree counterclockwise screen--cursor transformation) markedly increased. These findings confirm that the cerebellum and M1 have distinct functional roles in acquiring and retaining information during adaptive motor learning. Although assessing the effects of paired cerebellar and motor cortical tDCS in the same subject at the same time would have provided further interesting information, the study clarifies the different roles of the motor cortex and cerebellum in motor adaptation. More recently, Shah and co-workers (2013) assessed the effects induced by cerebellar tDCS vs. motor cortical tDCS on short-term ankle visuomotor learning. Subjects practiced a skilled visually-controlled ankle motor-tracking task while receiving anodal, cathodal or sham tDCS over the cerebellum, or over the M1. Anodal and cathodal cerebellar tDCS and anodal (but not cathodal) M1 stimulation improved ankle target-tracking accuracy (Figure 1). The lack of polarity-specificity reported in this study is not surprising given that anodal and cathodal tDCS reportedly have polarity-independent effects (Ferrucci *et al.*, 2008). As we explain in previous studies (Ferrucci et al 2008), a possible reason why cerebellar tDCS lacks polarity specificity comes from general physiological mechanisms (Lorente De No`, 1947): the loss of function in any excitable tissue can be obtained both with depolarisation and hyperpolarisation. For instance, classic neurophysiological experiments demonstrated that axonal conduction can be blocked, even for several hours, by depolarisation (‘‘depolarising’’ block) and also by hyperpolarisation

(‘‘hyperpolarising’’ or ‘‘anodal’’ block), both leading to the same decreased excitability and, ultimately, to a loss of function (Lorente De No`, 1947). Cerebellar tDCS of both polarities could interfere also with long-term depression (LTD) by altering the membrane potential fine-tuning needed for LTD in the cerebellar cortex. Whatever the mechanism, the study by Shah and co-workers (2013) further supports the conclusion that cerebellar tDCS improves visuomotor coordination for lower limb movements. Combining cerebellar and motor cortical stimulation might improve visuomotor learning even more effectively.

Using a relatively similar experimental approach, Dutta and co-workers (2014) assessed whether cerebellar tDCS influenced different types of voluntary visually-cued muscle activity in the tibialis anterior muscle. They found that anodal cerebellar tDCS increased the muscle activation latency in ballistic movements and reduced muscle activation learning related to visual-tracking sinusoidal movements (0.01 Hz) in the foot. Hence, in essence they found that anodal cerebellar DC stimulation worsened several variables related to voluntary visually-cued lower-limb muscle contraction in healthy subjects. The findings reported by Dutta and co-workers (2014) contradict those reported by Shah and co-workers (2013) who used a similar experimental setting for voluntary visually-cued muscle activation in the lower limb but at a faster frequency (0.2-0.4 Hz). Also, Shah and co-workers (2013) put the reference electrode over the ipsilateral buccinator muscle, whereas Dutta and co-workers (2014) put it on the contralateral forehead. Overall, the reported results suggest that cerebellar DC stimulation differentially influences visuomotor integration possibly in relation to the frequency of sinusoidal movements.

Current evidence points to the cerebellum as one of the structures that plays a critical role in motor memory acquisition (Criscimagna-Hemminger *et al.*, 2010; Donchin *et al.*, 2012). To investigate how the cerebellum and M1 contribute to human motor memory acquisition and retention in a paradigm to study motor memory (force field reaching task), Herzfeld and co-workers (Herzfeld *et al.*, 2014) compared the effects of cerebellar and M1 tDCS in 50 healthy

subjects. They found that cerebellar anodal stimulation enhanced error-dependent learning, whereas cathodal stimulation impaired it. They also showed that cathodal cerebellar tDCS during acquisition resulted in impaired retention as measured over 24 hours. These findings confirmed the critical cerebellar role in motor memory formation and retention.

A key to maintaining health in an older population consists in developing novel strategies against age-related deterioration in motor functions. In healthy older persons, age-related motor deterioration can depend on abnormal motor adaptation, a form of motor learning. Seeking a strategy for improving motor deterioration, Hardwick & Celnik (2014) assessed whether anodal cerebellar tDCS enhances adaptation in older subjects. Subjects had to make a “centre-out” reaching task, adapting to the sudden introduction of a visual cursor rotation. Participants sat in a robotic exoskeleton device with a monitor and vision of the hand and arm was occluded. They moved their arm to shoot a cursor from a start position through a circular target in 1 of 8 potential positions. Older subjects receiving sham tDCS were slower to adapt than younger subjects. But when older participants received anodal tDCS they adapted faster, similarly to younger subjects. These findings led Hardwick & Celnik to conclude that anodal cerebellar tDCS improves motor adaptation in older individuals and suggest cerebellar tDCS as a possible novel approach against age-related motor deficits.

In conclusion, overall available data show that cerebellar tDCS modulates several neurophysiological and behavioural motor variables in healthy subjects.

Non-Motor Functions

How and whether the cerebellum intervenes in non-motor functions remains controversial (Koziol & Lutz, 2013) but the available evidence suggest that cerebellar tDCS is emerging as a valuable tool in cognitive and psychophysiological research. The cerebellum is among the structures activated during a verbal working memory task (Kirschen *et al.*, 2005) and

cerebellar damage impairs working memory (Ravizza *et al.*, 2006). Prompted by this rationale, in the study from our laboratory that first described cerebellar tDCS (Ferrucci *et al.*, 2008), we observed that applying DC over the cerebellum influenced proficiency in the Sternberg task (a cognitive task assessing working memory). In the Sternberg task the subject has to remember whether a given number belongs to a sequence of numbers that previously appeared on a computer screen. We found that anodal cerebellar tDCS and cathodal cerebellar tDCS both impaired the practice-dependent improvement whereas tDCS over the DLPFC left it unchanged. This finding showed that the cerebellar tDCS-induced changes are structure-specific. Finally, cerebellar tDCS left visual evoked potentials unchanged, therefore ruling out visual cortex involvement. Substantially confirming our earlier findings (Ferrucci *et al.*, 2008) but using a slightly different methodology, Boehringer and co-workers (2012) showed that cathodal cerebellar tDCS blocked the practice-dependent increase in verbal working memory assessed with the digit span test, a task that requires the subject to remember progressively longer digit sequences read by the experimenter. They also reported that cerebellar tDCS induced no effects on word reading, finger tapping and a visually cued sensorimotor task. Hence, the available data consistently demonstrate that also when used with different experimental approaches, cerebellar tDCS alters the proficiency-related improvement in a working memory task, thus arguing in favour of a crucial cerebellar role in this physiological function and suggesting that cerebellar tDCS can be a valuable tool for manipulating working memory. Pursuing research on the cerebellar role in phonological storage and working memory, in a randomised, crossover and sham-controlled design, Macher and co-workers (2014) combined right cerebellar tDCS with functional magnetic resonance imaging (fMRI) to investigate how the human cerebellum contributes to encoding, maintenance, and retrieval of verbal information using a modified Sternberg task. After anodal, but not cathodal tDCS, the authors reported an impaired digit recognition capacity with an attenuated haemodynamic signal from the right cerebellar lobule VIIb together with weakened functional connectivity between this area and the posterior parietal cortex, during the late encoding phase. These findings suggest that the right

cerebellar lobule VIIb interacts with the posterior parietal cortex specifically during the late verbal encoding stages, when verbal information enters phonological storage. Hence they confirm previous results from our laboratory (Ferrucci *et al.*, 2008) and from Boehringer and co-workers (2012), expanding current knowledge to changes induced by cerebellar tDCS on phonological storage. The paper is important because it is the first to report combined findings from cerebellar tDCS and fMRI.

Cognitive tasks known to activate the cerebellum include those requiring attention (Strick *et al.*, 2009). Using cerebellar tDCS to modulate attention seemed an attractive possibility. Hence, to find out more about cerebellar control over working memory and attention, Pope and Miall (2012) used two cognitive tasks involving arithmetic skills of comparable motor difficulty but with different levels of cognitive complexity/load before and after cerebellar tDCS. They found that whereas the difficult task improved only after cathodal cerebellar DC stimulation, the easier task improved after anodal, cathodal or sham cerebellar tDCS. A further experiment in this paper assessed the effect of cerebellar DC stimulation on the ability to generate verbs, a language skill that some researchers attributed to the cerebellum (Fiez *et al.*, 1992). Cathodal cerebellar tDCS selectively facilitated the verb generation task. Overall this paper has two important implications. First, it shows that cathodal cerebellar tDCS selectively influences cognitive tasks with a high cognitive load related to memory and attention. And, from a practical point of view the improvement in the arithmetic test might be useful in considering new treatment strategies for dyscalculia. A second important point is that the work reports for the first time that cerebellar tDCS influences language. Though cerebral tDCS is widely used for studies on language in healthy subjects and patients with aphasia (Monti *et al.*, 2013), the observation arising from the study conducted by Pope and Miall suggests testing in aphasic patients tDCS concomitantly applied to the language areas and cerebellum.

Studies in healthy humans and in cerebellar patients underline the important role of the cerebellum in attention and in environmental exploration (Rondi-Reig & Burguiere, 2005; Baillieux *et al.*, 2008; Buckner, 2013; Reeber *et al.*, 2013). The cerebellum has also been implicated in recognizing salient changes in the environment by assessing mismatch negativity (MMN) in a group of cerebellar patients (Restuccia *et al.*, 2007). MMN is an event related potential component yielded by subtracting the EEG recording related to standard stimuli from the EEG recorded during odd stimuli. When Chen and co-workers (2014) assessed the MMN to auditory and sensory stimuli in 10 healthy subjects before and after cerebellar tDCS, they found that whereas anodal polarity increased, cathodal cerebellar tDCS decreased the amplitude of MMN evoked by somatosensory -- but not auditory stimuli. Although spontaneous, non event related EEG signal analysis, and -- again -- including stimulation to a control area would have provided a more clear understanding, the study indicates that by using cerebellar tDCS we can bidirectionally modulate (i.e. increasing or decreasing) the neurophysiological correlate of attention toward salient changes in the environment. This observation in healthy subjects might have implications for cognitive neurorehabilitation and for treating patients with attention deficit disorder.

Another important non-motor function controlled by the cerebellum is procedural learning (Koziol & Lutz, 2013). Functional neuroimaging studies demonstrated cerebellar activation during procedural learning tasks (Molinari *et al.*, 1997; Habas, 2010) and patients with cerebellar disorders often have impaired procedural learning performances. Under this rationale, in a later study we investigated whether cerebellar tDCS influences procedural learning as measured by the serial reaction time task (SRTT) (Ferrucci *et al.*, 2013). The SRTT is more than a simple motor learning task and has both motor and perceptual learning components (Robertson, 2007). During the SRTT the subject is required to mesh several different buttons on a keyboard after a target appears on a computer screen. Our main finding was that anodal cerebellar tDCS improved subjects' performance during procedural learning. Although this study, like many others, suffers from the lack of follow-up and

stimulation to a control area, it shows that the technique improves procedural learning but we have not demonstrate whether only perceptual learning components improved in the SRTT. This conclusion prompts further studies in patients with learning disorders.

Several experimental and some clinical observations argue that the cerebellum intervenes in affective control and in forming the associations between sensory stimuli and their emotional value (Strata *et al.*, 2011). For instance, in humans peri-vermian areas are activated for memory of personal emotional episodes and vermian damage influences the retention of fear memory. Hence, continuing research into non-motor cerebellar functions, we assessed whether cerebellar tDCS influences facial emotion recognition (Ferrucci *et al.*, 2012). Anodal and cathodal cerebellar tDCS significantly enhanced only the response to negative facial emotions. Cerebellar tDCS therefore influences the way healthy subjects recognise specific facial expressions thus showing that the cerebellum plays a direct role in recognising negative emotions. These findings suggest that cerebellar tDCS could be useful in patients with psychiatric disorders involving decreased cerebellar activation (Konarski *et al.*, 2005) and abnormal emotional recognition (Baillieux *et al.*, 2008).

Studies in Patients

Translating these basic research findings into clinical practice, Grimaldi and Manto (2013) tested anodal cerebellar tDCS in ataxic patients. They studied upper limb stretch reflexes (short-latency stretch reflexes SLSR; long-latency stretch reflexes LLSR), a coordination task, and computerized posturography. All these tests are abnormal in patients with cerebellar disorders. Cerebellar tDCS left SLSR amplitudes, coordination task and postural control unchanged, but significantly reduced LLSR amplitudes. The lack of effect upon coordination and posture suggests that cerebellar tDCS has no influence on the cerebello-cerebral networks sub-serving these functions (Figure 2). In a further study, the same authors (Grimaldi *et al.*, 2014) assessed the effects of a novel protocol using transcranial DC stimulation applied simultaneously to the motor cortex (cathodal) and cerebellum (anodal)

(cerebello-cerebral DC stimulation) on tremor, EMG activity and dysmetria in 2 patients with spinocerebellar ataxia (SCA) type 2. The rationale for using this paired cerebro-cerebellar stimulation arises from the observation that cathodal motor cortical DC stimulation improves some features related to a cerebellar deficit (Pozzi *et al.*, 2014) and hence combined stimulation applied to the brain and cerebellum should double the beneficial effects. Yet, Grimaldi and co-workers (2014) found that transcranial cerebello-cerebral DC stimulation reduced tremor, hypermetria and the latency of the antagonist EMG activity. Although the study fails to clarify the individual roles of stimulation over the cerebellum or the motor cortex in inducing the reported clinical improvement and the results need to be replicated in larger controlled studies, the observation suggests a new therapeutic option for patients with cerebellar ataxia. Future clinical research work should systematically assess the patients' features predicting an optimal response and investigate how to induce a long-lasting clinical effect to durably improve the patient's quality of life.

Cerebellar tDCS Modelling Studies

The effects of transcranial DC stimulation depend on the electric field and current density field distributions produced in the nervous tissue. Their knowledge is therefore important to predict the location and extent of the stimulated region as well as the stimulation intensity in a specific region of the CNS. At present, these distributions are best-estimated by computational models (Peterchev *et al.*, 2012). For transcranial electrical stimulation, the quasi-static regime can be applied, and hence the electric field E can be given by the negative gradient of the electric potential φ (i.e. $\mathbf{E} = -\nabla\varphi$) , whereas the current density J is obtained from the electric field E by means of the relation $\mathbf{J} = \sigma\mathbf{E}$, where σ is the electric conductivity in the tissue. The distribution of the potential φ inside the conductive medium (i.e. the head) is obtained by solving the current continuity equation ($\nabla \cdot \mathbf{J} = -\nabla \cdot (\sigma\nabla\varphi) = 0$), subjected to the appropriate boundary conditions. In simple cases, the equation can usually

be solved analytically but when the volume conductor is geometrically complex numerical methods are needed. Based on this theory, the literature has proposed different computational models for transcranial electrical stimulation in which the head representation ranged in complexity from concentric sphere models to more detailed, simplified geometric representations up to high-resolution, MRI-derived models incorporating complex tissue geometries. Because the tissue properties are poorly known, researchers have used different conductivity values mainly derived from static resistivity measures or extrapolated from 10 Hz data, and have sometimes even included tissue conductivity anisotropy (Bikson *et al.*, 2012; Peterchev *et al.*, 2012; Ruffini *et al.*, 2013). Close to the stimulation site, according to data calculated using realistic head models, the maximum electric field magnitude in the grey matter was about 0.2–1.5 V/m for a 1 mA current applied through large electrodes (25–35 cm^2) (Ruffini *et al.*, 2013). Overall, modelling studies were useful in interpreting and optimising stimulation outcomes, but an important question is how to validate the electric field calculations experimentally, because empirical data on the current density in the brain during tDCS are largely missing. In early research, Dymond and co-workers (1975) reported electric field values of 0.6–1.6 V/m for a current intensity of 1 mA. In their experiment, the stimulation electrodes were placed bilaterally over the frontal pole and the mastoids and the recording electrodes were implanted near the hippocampus. Findings from these models therefore need interpreting with caution. This caveat relates especially to assumptions on tissue conductivities or the accuracy and precision of the segmentation of different tissues (i.e. the tissue masks) from high-resolution anatomical data. Indeed, the number and precision of the tissue masks obtained may influence predicted current flow. In this context, only two modelling studies have been specifically designed for cerebellar tDCS. Mimicking the electrode montage used by Ferrucci and co-workers (Ferrucci *et al.*, 2008; Ferrucci *et al.*, 2012), a modelling study (Parazzini *et al.*, 2014b) using MRI-derived models for three subjects predicted that using one conventional sponge-tDCS electrode placed over the cerebellum and an extra-cephalic electrode over the right arm concentrate the current flow mainly to the cerebellum, with a slight spread to other structures. This result gave

support to previous experimental observations that cerebellar tDCS failed to influence visual evoked potentials (Ferrucci *et al.*, 2008) therefore excluding stimulation to the visual cortex. Additionally, the study showed that individual anatomical variability somehow influences electrical field distributions: the electrical field spreads more toward the brainstem tegmentum in the child model than in adult models (Figure 3). Because experiments with cerebellar tDCS are still lacking in children nor do we know whether the possible brainstem spread is functionally relevant, the use in paediatric age must be conservatively considered potentially dangerous.

In line with the conclusion by Parazzini and co-workers (2014b), a preliminary modelling study with high-definition tDCS electrodes (1 cm in diameter) (Rahman *et al.*, 2014) confirmed that the applied current reaches the cerebellum. Because the electrode montages used by Rahman have never been tested in humans, their hypothesis remains questionable. Also, other important variables for cerebellar tDCS that need to be systematically tested in future studies include cerebrospinal fluid current shunting, electrical field funnelling by fissures or holes in the skull, and different conductivities in grey and white matter. Collectively, however, computational models for cerebellar tDCS result in an electric field in the cerebellum with a maximum ranging between 0.2–3.5 V/m for a 2 mA applied current density. These values, as well as being in the same range as the weak electric field found for brain stimulation, are of the same order of magnitude as previous experimental results. In experiments using isolated turtle cerebellar cells, Chan and co-workers found that the threshold for modulating both Purkinje and stellate cells was around 15-20 V/m (Chan & Nicholson, 1986). In a later study, Chan and co-workers (1988) predicted that Purkinje cells will polarise by 0.2 mV per 1 V/m applied electric field. This polarisation, albeit small, can affect the firing rate for large neuronal populations (Frohlich & McCormick, 2010). In mammalian brain, Jefferys (1981) reported a threshold of 5-10 V/m for granule cells in the hippocampal slice, whereas in the crayfish stretch receptor Terzuolo and co-workers (1956) reported a 1 V/m threshold for modulating active neurons. Several years later, Ghai and co-workers (2000) reported that electric fields as low as 1 V/m could modify neuronal activity in

hippocampal slices, and Francis and co-workers (2003) provided the experimental evidence that neuronal networks are sensitive to electric fields lower than 1 V/m. Hence, overall, available experimental data indicate a threshold for neuronal interactions with the electric field between 1 and 20 V/m and therefore in the range of those estimated by cerebellar tDCS models.

Cerebellar tDCS Mechanisms of Action and Safety

Because studies on cerebellar tDCS began only recently few data are available about its mechanisms of action. tDCS could basically act at two time-points: first, when the electric field is applied, and second after DC offset.

Though some general principles underlying changes induced by the electric field on the CNS may apply also to the cerebellum, given its intrinsic passive electrical properties this brain area could respond differently to electricity. For instance, permittivity is higher in mouse cerebellum than in the brain or brainstem and conductivity is higher at frequencies below 1000 MHz (Nightingale *et al.*, 1983). Studies on conductivity in the cat cerebellar cortex show also that the various cortical layers differ in isotropy the granular layer being more isotropic than other layers (Yedlin *et al.*, 1974). All these differences, particularly those related to conductivity, can be important given that tissue dielectric properties play a key role in electric field computation and the current density distributions during transcranial stimulation, as shown by the foregoing equations (see previous section Cerebellar tDCS Modelling Studies).

Once the electric field reaches the cerebellum it can induce functional changes. Current experimental knowledge provides no precise information on where cerebellar tDCS-induced changes take place (cerebellar cortex, deep nuclei, white matter). Nor does it specify whether they involve one cerebellar area alone or the whole cerebellum. Cerebellar tDCS could interfere with membrane polarisation in Purkinje cells and in other neurons, fibres

(mossy fibres and climbing fibres) and glial cells. DC stimulation applied to the cerebellar cortex in the decerebrated cat influences Purkinje and granular cell activity in a polarity-specific manner: whereas anodal DC (0.1-1 mA) flowing in the dendrite-axonal direction increased tonic neuronal activity, cathodal DC decreased it (Brookhart *et al.*, 1952). Similar more recently reported findings showed that when an electric field is applied across the turtle cerebellum it elicits differential effects in various cerebellar neurones according to its relative orientation (Chan & Nicholson, 1986). Current flowing from the cortical surface to the fourth ventricle predominantly excites Purkinje cells and some stellate cells.

Two main physiological issues about cerebellar tDCS require further research. The first is whether the induced effects are polarity-dependent. Whereas some experiments found that anodal and cathodal cerebellar tDCS elicit the same effects (Ferrucci *et al.*, 2008; Ferrucci *et al.*, 2012; Hamada *et al.*, 2012) on a given task or function, others reported polarity-specific changes (Galea *et al.*, 2009; Jayaram *et al.*, 2012; Pope & Miall, 2012; Grimaldi & Manto, 2013). Considering that different cerebellar areas are implicated in various functions, and that neurons in all these areas are variously oriented in relation to the applied electric field, the lack of polarity specificity is hardly surprising because it again underlines that the induced changes in excitability depend on the electric field's direction. And equally important, classic neurophysiological experiments on axonal excitability have shown that both polarities can block action potential propagation (Lorente De No`, 1947). Overall, current knowledge therefore shows that the human cerebellum responds to cerebellar tDCS in a complex manner, possibly depending on the function studied, the task used, the electric field geometry and orientation, and its strength or duration. Another important point to understand about how or where cerebellar tDCS acts in the brain is that it can induce lateralized effects. Whereas in some experiments from our laboratory we used a stimulating electrode covering the whole the cerebellum (Ferrucci *et al.*, 2008; Ferrucci *et al.*, 2012; Ferrucci *et al.*, 2013), others placed electrodes to stimulate the hemi-cerebellum and reported highly side-specific cerebellar tDCS-induced changes (Galea *et al.*, 2009; Jayaram *et al.*, 2012).

We also need to know what happens in the cerebellum after current flow ceases. Because transmembrane polarisation lasting only a few minutes induces prolonged spiking activity in Golgi inhibitory cerebellar neurons (Hull *et al.*, 2013), Golgi-cell activity could partly explain cerebellar tDCS after-effects. The mechanisms of action underlying cerebellar DC stimulation could also involve ionic gradients in the extra-cellular space, specific cellular inactivation or activation (including protein synthesis, gene expression, and channel-pump inactivation) common to several cell types (including glia, and smooth muscle cells in cerebellar vessels). Other mechanisms could also involve receptors and neurotransmitters. Whereas electrical phenomena probably initiate cerebellar tDCS-induced changes, other mechanisms related partly to non-electrical phenomena could intervene to maintain them. For instance, cerebellar neurotransmitters such as myoinositol (Goto & Mikoshiba, 2011), GABA and glutamate (Ottersen, 1993), undergo substantial changes in the brain after brain tDCS (Rango *et al.*, 2008; Stagg *et al.*, 2009), and may do the same in the cerebellum after cerebellar tDCS. Because cerebellar stimulation also modulates dopamine release in the basal ganglia (Nieoullon *et al.*, 1978) this mechanism could also apply for cerebellar tDCS. Hence cerebellar tDCS-induced neurotransmitter changes could help to explain how cerebellar DC stimulation acts.

Theoretical estimations (Parazzini *et al.*, 2014b) and experimental data (Galea *et al.*, 2009) imply that cerebellar tDCS has no significant functional effects on the brainstem in adults. Modelling studies (Parazzini *et al.*, 2014b; Rahman *et al.*, 2014) demonstrate that the electric field centres mainly in the cerebellum and no research groups have reported adverse effects after or during cerebellar tDCS in adults.

Clinical Perspectives

Available data provide evidence that cerebellar tDCS modulates human cognitive and motor cerebellar functions in healthy subjects and in the few patients assessed but further studies

need to confirm these findings, find out how cerebellar tDCS works and develop optimum stimulation settings and protocols in patients with neurologic and psychiatric disorders. Research findings already provide evidence that cerebellar tDCS can influence motor adaptation, learning, memory and emotional processing in healthy humans and these changes could be clinically important in patients with various disorders–involving cerebellar dysfunction and possibly also as a strategy against motor aging in the elderly. Future studies in patients should, however, take into account evidence that the cerebellar nuclei can be variably affected by the disease and, among other variables, carefully consider the lesion's location in the cerebellar circuitry, and possible extra-cerebellar lesions.

Transcutaneous Spinal Cord DC Stimulation (spinal tDCS).

Single-pulse TMS to the cervical and lumbar spine has been widely used to assess central and peripheral motor conduction time in clinical neurophysiology, by evaluating evoked compound muscle action potentials following motor root activation at the neuroforaminal level (Ugawa *et al.*, 1989; Knikou, 2013). Conversely, few studies have assessed whether repetitive spinal magnetic stimulation can modulate spinal cord functions. A recently published review addressed the effects induced by repetitive spinal TMS on motor control (Beaulieu & Schneider, 2013), whereas few studies have described effects on pain syndromes (Smania *et al.*, 2003; Krause *et al.*, 2005). Spinal tDCS occurs to have some practical advantages on rTMS.

In applying spinal tDCS, researchers used certain key technical features. For lumbar spinal cord modulation, the active electrode (measuring about 5 x 7 cm) was usually placed over the spinous process of the tenth thoracic vertebra and the reference above the right shoulder (Cogiamanian *et al.*, 2008; Cogiamanian *et al.*, 2011; Lamy *et al.*, 2012; Lamy & Boakye, 2013). For cervical spinal cord modulation, the active electrode was positioned on the seventh cervical vertebra and the reference on the anterior part of the neck (Lim & Shin, 2011). Another probably influential variable that needs to be systematically investigated

when applying spinal tDCS is whether the reference electrode is positioned on the arm or elsewhere. Also, stimulation intensity and duration were kept relatively constant across the various studies from different groups: intensities between 2 and 2.5 mA were usually applied for 15/20 minutes (Table 2, part A). Spinal DC often elicits short-lasting tingling sensations when stimulation begins and ends and sometimes redness under the electrode.

Studies in Healthy Humans

Experiments in humans have focused on ascending tracts, descending tracts and spinal reflexes (Table 2, part A). In an earlier study, Cogiamanian and co-workers (2008) who first described the technique assessed the after-effects induced by anodal and cathodal spinal tDCS on somatosensory potentials (SEPs) before and after applying spinal tDCS. SEPs assess the lemniscal pathways (Cruccu *et al.*, 2008). Anodal spinal tDCS selectively reduced the cervico-medullary SEP amplitudes for at least 20 min after stimulation offset, whereas cathodal tDCS left all SEP components unchanged (Figure 4) thus demonstrating an effect on the lemniscal system. In a similar way, Truini and co-workers (2011) investigated the after-effects of anodal spinal tDCS and cathodal tDCS on laser evoked potentials (LEPs): the scalp potentials elicited by peripheral laser stimulation reflect mainly peripheral Aδ fibre activation (Romaniello *et al.*, 2003). These fibres transmit pain information to the brain through the spinothalamic tract. Anodal spinal tDCS reduced LEP amplitudes evoked by foot laser stimulation whereas cathodal tDCS failed to induce attenuation (Figure 5) and therefore spinothalamic system activity. Hence these two studies show that spinal tDCS modulates at least two ascending sensory pathways in humans, regardless of where the tract lies anatomically on the transverse spinal cord plane: the lemniscal pathway lies posteriorly whereas the spinothalamic tract is ventral and lateral (Bican *et al.*, 2013). This observation therefore implies that spinal DC stimulation can also influence other tracts, including corticospinal fibres. To test the corticospinal hypothesis, Lim and Shin (2011) assessed motor evoked potentials elicited by TMS to the motor cortex in the upper limb before and after cervical spinal tDCS. They reported that spinal tDCS increased

corticospinal excitability in a polarity-independent way, eliciting no effects on the H-reflex (Lim & Shin, 2011). Though interesting, these results are difficult to interpret both because the investigators used a unique experimental set up (stimulating electrode on C7, reference electrode on the anterior part of the neck) not comparable to the available studies on the thoracic spinal cord and because polarising currents could spread to the brachial plexus. Besides, the different polarity surprisingly has no effect on the direction of the induced effect (excitation or inhibition). A possible explanation for the lack of polarity specificity might reside in the electrode position generating an electric field crossing the spinal cord differing by 90° in orientation from the studies by Cogiamanian and co-workers (2008) and Truini and co-workers (2011). As happens within other CNS structures, knowing how the electric field is oriented with respect to cell morphology is critical to describe cellular polarisation and the associated functional effects.

The DC induced effects on segmental circuitries were mainly assessed on the Ia-motorneuronal connections by recording the H-reflex. Although some studies, not being specifically designed to assess spinal cord modulation at segmental level (Cogiamanian *et al.*, 2011; Lim & Shin, 2011), found no spinal tDCS-induced changes in H-reflex basic features (latency, threshold, Hmax/Mmax ratio), they could not totally exclude a segmental effect. The first study specifically designed to investigate more complex effects at segmental level dates back to 2010. In a protocol designed to investigate spinal DC effects on H-reflex homosynaptic depression (i.e. the progressive H-reflex depression following repetitive H-reflex nerve stimulation at certain frequencies), Winkler and co-workers (2010) showed that even though a simple H-reflex excitability measure (the Hmax/Mmax) remained unchanged, anodal spinal tDCS induced a long-lasting decrease in homosynaptic depression whereas cathodal spinal tDCS increased it. The investigators suggested that the lack of Hmax/Mmax ratio modulation indicates that spinal tDCS had no significant influence on alpha-motorneuron excitability, and that its effect on homosynaptic depression arises from the spinal tDCS-induced changes on the Ia-motorneuron connections. Whatever the

mechanisms, given that homosynaptic depression is decreased in spastic patients (Grey *et al.*, 2008), spinal tDCS might be a promising tool to improve spasticity. Providing more information on how spinal tDCS alters spinal cord plasticity, Lamy and co-workers (2013) showed that anodal spinal tDCS induced a leftward shift in the soleus H-reflex stimulus-response curve (i.e. increased excitability) lasting up to 15 minutes after stimulation ended, whereas cathodal and sham stimulation left the curve unchanged. A subsequent study (Figure 7) confirmed both findings (Lamy & Boakye, 2013). In accordance with previous observations, Lamy and co-workers confirmed that the Hmax/Mmax ratio was unaffected by spinal tDCS. Hence, given that spinal DC leaves the basic H-Reflex features (amplitude, area, latency and Hmax/Mmax ratio) unaffected these measurements are probably unreliable for assessing DC-induced changes in Ia-motorneuron synaptic plasticity. A final interesting observation is that the effect of spinal tDCS on Ia-motorneuronal connections is related also to the stimulated subject's genetic features. Assessing whether the brain-derived neurotrophic factor (BDNF) polymorphism influenced changes in the soleus H-reflex recruitment curve induced by anodal spinal tDCS, Lamy and co-workers (2013) found that the methodology was effective only in BDNF Val homozygotes (Lamy & Boakye, 2013). The study is novel because it correlated the genotype with the response to the electric field and suggests a genetically-based interindividual variability in the effect of spinal (and cerebellar?) tDCS.

Continuing neurophysiological studies on reflexes, Cogiamanian and co-workers (2011) evaluated changes in the lower limb polysynaptic flexion reflex (LL-Fr), a response that assesses nociceptive pathways, and the effect of various treatments on pain (Cruccu *et al.*, 2004). The Fr late response is a high-threshold nociceptive Aδ fibre mediated reflex and its threshold corresponds to the pain threshold, whereas the reflex size is related to the pain perception level (Sandrini *et al.*, 2005). Anodal spinal tDCS induced a long-lasting Fr depression and reduced the RIII area (Figure 6) thus demonstrating that spinal tDCS can decrease the nociceptive reflex and suggesting its possible analgesic effect. Nonetheless,

although the LL-Fr is a polysynaptic and multisegmental spinal response, supraspinal control exerts an important role in modulating this pain reflex. Thus, a possible descending pathway modulation cannot be excluded. In addition, because withdrawal reflexes are influenced by the cognitive state (Bjerre *et al.*, 2011), further studies should account for a possible correlation between cognitive activity and spinal withdrawal reflex modulation. The effects induced by spinal tDCS on laser-evoked potentials (Truini *et al.*, 2011) and on the Fr-LL (Cogiamanian *et al.*, 2011), overall imply that this technique might have a therapeutic role in managing pain.

Studies in Animals

In contrast to research on cerebellar DC stimulation, researchers in the past few years started studying the effects of spinal DC stimulation in the animal model using healthy animals and animals with spinal cord injury.

Aguilar and co-workers (2011) investigated how spinal direct current stimulation delivered at thoracic level influences spontaneous activity and SEPs in the gracile nucleus and primary somatosensory cortex in urethane-anaesthetised rats. The stimulating electrode (total contact surface 0.785 cm^2) was placed on the thoracic spinal cord over the dura mater, whereas the return electrode was placed under the skin in the anterior abdominal area. Although this electrode configuration was used to maximise the electrical field across the spinal cord, this orthogonal arrangement could have altered the electric field orientation with respect to the spinal cord, so that the transverse component predominated over the longitudinal one. Intensity was set to 1 mA (current density 1.27 mA/cm^2) for 15 minutes. In the gracile nucleus anodal spinal tDCS increased single-unit activity but decreased the size of the somatosensory evoked potentials, and cathodal spinal tDCS did the opposite. In addition, anodal tDCS desynchronized activity in the rat somatosensory cortex, whereas cathodal tDCS hyper-synchronised it. An important point in interpreting these results is that the invasive electrode arrangement they used generates far higher current density than that estimated in human spinal cord during non-invasive stimulation (Parazzini *et al.*, 2014a).

After the foregoing study assessing the somatosensory system, to evaluate the corticospinal connections, Ahmed (2011) explored in mice the effects of spinal DC stimulation on motor responses evoked by cortical stimulation in triceps surae muscle. He found that anodal current depressed the triceps surae muscle twitch force for the cortically-evoked motor responses with a rebound facilitation persisting 20 minutes after the current offset, and vice-versa with cathodal stimulation. Anodal spinal tDCS increased the latency of the cortically-evoked tibial nerve potential during current flow with a rebound latency shortening after current offset, and vice-versa with cathodal spinal stimulation. Ahmed concluded that the observed effects could reflect changes induced by the electric field on descending pathways or spinal motorneuronal excitability. Ahmed also described the effects of anodal and cathodal spinal tDCS matched with repetitive cortical stimulation on the responses elicited by single cortical shocks. He proposed that repetitive cortical stimulation induced persistent excitability changes and that concomitant repetitive cortical stimulation had no influence on the effects induced by spinal tDCS. In contrast, concomitant repetitive cortical stimulation boosted the cathodal tDCS effects. He therefore concluded that anodal and cathodal spinal DC stimulation act through different mechanisms. In a further group of experiments in anaesthetized mice (Ahmed, 2013b), Ahmed found that cathodal tDCS enhances the simple and complex behavioural motor responses elicited by electrical stimulation delivered in trains over the hind-limb representation in the motor cortex. Ahmed also found that cathodal spinal DC stimulation increased cortically-elicited ankle and multijoint movements. In the same paper he also tested the effects of cathodal spinal DC stimulation on the spinal circuit activity induced by glycine and GABA receptor blockers (picrotoxin at variable concentrations, mixed with strychnine), and reported that receptor-blocker modulation induced bursting spinal activity, thus concluding that spinal DC stimulation facilitates cortically evoked motor responses by acting on spinal circuits.

Other experiments aimed also to assess the neurophysiological effects on spinal motorneurones, on segmental Ia-motorneuronal connections, and the neurochemical

changes induced by spinal DC stimulation. Ahmed (2011) reported that spontaneous activity in the tibial nerve increased more after anodal than after cathodal spinal tDCS (intensities from 0.5 mA to 3 mA, 30-second steps for 3 minutes, maximal current density 38.22 A/m^2) during current flow but not after its offset. Cathodal DC stimulation increased the H reflex size (Ahmed & Wieraszko, 2012). Conducting a series of studies on spinal tDCS-induced neurochemical effects, Ahmed (2011) also compared the activity induced in the tibial nerve by anodal and cathodal spinal DC stimulation with the activity induced by injecting GABA and a glycine receptor blocker into the spinal cord in two mice. The activity induced by GABA and glycine blockers resembled the activity induced by cathodal spinal stimulation, thus suggesting that the mechanism underlying the action of cathodal stimulation in mice involves GABA and glycine receptors. In a further work Ahmed (2013b) also tested the effects of cathodal spinal DC stimulation on the motorneuronal bursting activity induced by picrotoxin and strychnine injected into the spinal cord (see previous experiment). Continuing research on the neurochemical effects of spinal DC stimulation Ahmed and Wieraszko (2012) studied whether this technique could induce release of the glutamate analogue aspartate. Using a special custom-built chamber they evaluated the amount of aspartate released by isolated mouse spinal cord after spinal DC stimulation and found that cathodal spinal DC stimulation increased aspartate, whereas anodal DC reduced aspartate concentrations therefore concluding that effects of spinal DC stimulation in mice at least partly depend on glutamate changes. In conclusion, neurochemical studies overall suggest that spinal DC stimulation in mice induces its effects partly through the neurotransmitters GABA, glycine and the glutamate analogue aspartate.

Despite their interest, studies in animals have some limitations. First, the anaesthetic drugs used during experiments can influence spinal cord excitability. In particular, ketamine-induced effects on spinal cord vary from animal to animal: for example, ketamine reduces synaptic transmission at excitatory interneuron terminals in the cat spinal cord (Lodge & Anis, 1984), whereas its effect on rat spinal cord seems related to the anaesthesia level

(Zandieh *et al.*, 2003). Theoretically, spinal tDCS can exert a different effect on spinal cord functions under different anaesthetic drugs and levels. Second, the results in animals are difficult to compare with those in humans for technical reasons: among other details, electrodes are positioned subcutaneously, hence modifying the electrical field distribution through non-neural tissues and finally increasing the current density directly applied on the spinal cord. Equally important, the electrode position differs from the configuration used in human experiments (and consequently the electrical field differs in orientation), stimulation duration is shorter (seconds in rats, minutes in humans) and the applied current density is much higher in animal experiments than in humans. Finally, because animals are smaller than humans, changes induced by spinal DC stimulation could distinctly differ in animals and humans. Thus the effects of spinal tDCS in animals and humans are only partially comparable.

Studies in Animals and Patients With Spinal Cord Injury

Because spinal cord injury is a dramatic emergency that usually defies treatment, any therapeutic option to influence the outcome in patients would be extremely helpful. Under this premise, Ahmed conducted several experiments in spinal cord injured mice. He assessed the behavioural effects induced by spinal DC stimulation and repetitive cortical and sciatic stimulation combined and found that combining the two techniques improved walking recovery in lesioned animals. The reciprocal potentiating effects of cathodal spinal DC stimulation and repetitive cortical stimulation on motor responses evoked by single cortical shocks are present also in animals with incomplete spinal cord injury and contusions (Ahmed & Wieraszko, 2012). In these mice the response amplification obtained by combining the two techniques lasted at least 90 minutes after the stimulation offset, indicating an interesting new direction for research in patients with SCI. Continuing research on a mouse SCI model Ahmed (2013a) assessed whether spinal tDCS increases recovery. To do so he used a complex experimental design based on combining the two stimulation techniques (cortico-sciatic stimulation and spinal tDCS) so as to increase the cortically-

evoked motor responses and found that cathodal spinal DC stimulation further increased the motor responses evoked by cortico-sciatic stimulation in SCI mice. A limitation of the experiments in rats with SCI is that spinal cord lesions evolve over time and spinal circuits change according to the time elapsed after the injury. Spinal tDCS could modulate spinal circuitries in the acute, subacute and chronic phases through different mechanisms and, direct currents could contribute to facilitate spinal cord healing, as already shown in “in-vitro” experiments (Haan & Song, 2014).

Ahmed’s study in SCI mice prompted researchers to ask whether spinal tDCS could have some effect in SCI patients. To answer this question, Hubli and co-workers (2013) applied spinal tDCS in patients undergoing neurorehabilitation after SCI. They evaluated whether spinal tDCS or assisted locomotion using driven gait orthosis (Lokomat®) enhanced excitability (measured using the spinal reflex) in spinal circuits underlying locomotion in patients with complete SCI (Hubli *et al.*, 2013). In line with the previous observations on spinal reflex modulation in healthy subjects (Winkler *et al.*, 2010), anodal spinal tDCS and assisted locomotion in patients increased SR amplitude, whereas cathodal and sham stimulation left it unchanged. This finding shows that spinal tDCS can elicit functional effects also when the spinal cord is detached from the rest of the CNS. Hence, in patients with complete SCI, spinal direct current stimulation acts through purely spinal mechanisms. These findings open an important avenue of research designed to rescue residual spinal functions in SCI patients (Hubli *et al.*, 2013).

Spinal tDCS Modelling Study

As it did for the cerebellum, a modelling approach could provide determinant information about the effect and mechanism of action underlying spinal tDCS. Continuing their research in the field, Parazzini and co-workers (2014a) conducted the first modelling study aiming to estimate the current density distributions in the spinal cord in various human models (Figure 3). They modelled three electrode montages, with the anode always over the spinal process

of the tenth thoracic vertebra and the cathode placed: A) above the right arm; B) over the umbilicus; and C) over the head vertex (in the Cz location according to the 10-20 EEG system). Despite some inter-individual differences due to anatomical variability, within the spinal cord the current density (and the electric field) tended to be primarily directed longitudinally along almost all the vertebral column, particularly with a reference electrode over the right arm and over Cz. On transverse spinal cord sections at thoracic level, the current density distributed uniformly. This finding suggests that the ventral (motor) and dorsal (sensory) axonal tracts undergo identical electric field strength. The reference electrode position had an effect also on the spinal cord region where the current density distribution is higher: for instance, the maximum current density at thoracic level was about 0.016 A/m^2 with the reference electrode on the right deltoid muscle. Experiments testing the other electrode positions yielded an even higher maximum current density if the reference electrode was placed on the umbilicus or over Cz (0.018 A/m^2 at lumbar level and 0.075 A/m^2 at cervical level). Across the electrode montages and human models, the maximum electric field ranged between 0.9-4.5 V/m per 3 mA; these values are of the same order of magnitude as the values found for brain tDCS. The current density distributions with a reference electrode over the right arm demonstrate that this montage acts mainly at thoracic level with minimal current spread. Conversely, the field distributions with the reference electrode over Cz show that it acts also supraspinally at bulbar level. Spinal tDCS with the reference electrode over Cz could double its physiological effects by doubling the site of action: one located at spinal level and the other at brainstem or cortical level. Hence according to the experimenter's objective, if the aim is to focus on the region for spinal modulation, the reference electrode should be positioned as close as possible to the anode so as to minimize the longitudinal spread of the induced field (as shown for example with the montage on the right arm to focus on the thoracic level). Conversely, moving the electrode to a more distant position will involve larger spinal cord regions and potentially even regions in the brain or brainstem (as shown for example with montage on Cz).

As expected, owing to the distance between the electrode and the spine, the models show that even if spinal tDCS generates current that reaches the spinal cord, current disperses also in surrounding tissues, mainly the muscles on the back and the nerve roots. This spread is slightly higher in younger models. A side to the limitation discussed before, the results from this modelling study can be useful in translating animal results to humans. First, human studies used lower current density applied on the skin, whereas animal studies mainly used higher current density directly applied over the spinal cord or subcutaneously, thereby substantially differing in current magnitude in the spinal cord. Second, the different body size and electrode arrangements between animals and human studies could change spatial distribution of the electric field (and current density) in the spinal cord, thereby possibly influencing polarisation. Other important variables for spinal tDCS that need to be systematically tested in future modelling and esperimental studies include cerebrospinal fluid current shunting, electric field funnelling by fissures or holes in the spine, and different conductivities in grey and white matter.

Spinal tDCS Mechanisms of Action and Safety

Overall, whereas in humans anodal spinal tDCS suppresses responses mediated by spinal ascending pathways and somehow enhances segmental reflex circuit function, cathodal spinal tDCS enhances responses mediated by spinal ascending pathways and suppresses segmental reflex function. Experiments in animals substantially confirmed that spinal tDCS acts on ascending/descending spinal pathways and on segmental reflex responses, suggesting glutamatergic, GABAergic and glycinergic system involvement and effects on spinal plasticity. Given the distance between the spinal cord and the skin surface the major changes induced by spinal tDCS might seem surprising but current could easily flow within the spinal canal through the intervertebral spaces. As they do for the cerebellum, the spinal DC-induced changes in spinal cord excitability take place at two key time-points, during (online effects) and after spinal tDCS ends (after-effects). The online effects on a neuron/axon exposed to an applied electrical field depend on several features ranging from

field properties (intensity, polarity and direction), to neuroanatomical and neurophysiological properties in the targeted spinal structure (Elbasiouny & Mushahwar, 2007). One of the first reports describing how polarising current influences spinal circuitries (Eccles *et al.*, 1962) investigated changes in primary afferent fibre excitability in cats. A dorsal-ventral current hyperpolarised the presynaptic terminals in primary afferent fibres, whereas a ventral-dorsal current did the opposite. Changes in membrane potentials were also associated with changes in trans-synaptic (Eccles *et al.*, 1962) or direct alpha motorneuron excitability (Hounsgaard & Kiehn, 1993). The electrical field modulates voltage-sensitive calcium channels in turtle spinal motorneuron dendrites, modifying the total calcium inflow in the motorneuron (Hounsgaard & Kiehn, 1993).

How polarizing currents change neuronal membrane excitability depends on how spinal cord fibres are spatially oriented in relation to the electric field (Terzuolo & Bullock, 1956). Hence, a given electrical field polarity can increase spinal tract excitability in the white matter and at the same time decrease excitability in neural elements in the grey matter or vice-versa. This mechanism can explain why anodal tDCS inhibits transmission in the ascending spinal pathways (Cogiamanian *et al.*, 2008), whereas it increases H-reflex excitability (Lamy *et al.*, 2012).

Spinal tDCS is a non-invasive technique considered safe in adults. No published studies have reported adverse effects after spinal tDCS. In humans, Cogiamanian and co-workers (2008) failed to report changes in serum neuron-specific enolase (a marker of neuronal damage) before and after stimulation offset. In rats, Ahmed showed that spinal tDCS left spinal cord integrity unchanged (Ahmed, 2011). Another modelling study (McCreery *et al.*, 1990) suggested that current density levels found in the spinal cord (see section spinal tDCS modelling study) are well below the threshold for neural tissue damage (McCreery *et al.*, 1990). Modelling data (Parazzini *et al.*, 2014a), suggest, however, that current spread

towards other anatomical structures is slightly higher in small subjects and children than in adults. Hence spinal tDCS must be used cautiously in children or small subjects.

Clinical Perspectives

Available data show that spinal tDCS modulates spinal cord excitability and function, and suggest that these neuromodulatory changes might be used to improve outcome in patients with neurological disorders and SCI (Hubli *et al.*, 2013) in different ways. First, SCI can be considered as a disconnection syndrome, in which the segmental circuitries below the lesion are abnormally activated owing to defective supraspinal control. Spinal DC stimulation might therefore help to restore their normal activation by reducing excitability in the hyperactivated circuit and vice-versa. A further important point is that electric fields are used in research to promote regeneration and repair interrupted nerve fibres in SCI (Hamid & Hayek, 2008). Hence a technique that could non-invasively generate an electric field able to reach the injured spinal cord in patients might lead to a new therapeutic option aiming to promote axonal regeneration. Finally, in clinical practice patients can easily undergo repetitive spinal tDCS sessions (i.e., daily sessions for more than two consecutive days increasing the total charge applied) and outpatients (or their caregivers) can be trained to use this technique at the patient's home, allowing patients to be treated in large numbers.

Concluding Remarks

Weak DC delivered transcutaneously in humans over the cerebellum and over the spinal cord for minutes can elicit prolonged changes in neurophysiological and behavioural responses related to cerebellar and spinal functions. The electric field generated by cerebellar tDCS reaches the cerebellum, whereas the electric field for spinal tDCS reaches the spinal cord. The physiological effects elicited by both techniques arise from functional changes in the stimulated structure (cerebellum or spinal cord) though no evidence yet rules

out possible (transynaptic or antidromic) changes in other brain or brainstem structures triggered by changes in the primary target structure. Several effects probably underlie DC-induced neuroplasticity and possibly neurotransmitter changes. Although no experimental data are available in children, experimental works failed to report adverse effects in adults.

Preliminary data suggest that both cerebellar tDCS and spinal tDCS can improve some physiological variables in selected patients and animals with experimental spinal cord injuries. Much work remains to be done to confirm the data, to understand in pathological conditions the possible mechanisms of action underlying these two techniques also at cellular level and how to apply them. For example, we need to investigate changes induced by repeated stimulation sessions, compare stimulating electrode montages, examine how body size and age could influence results, and study possible interactions with ongoing drug treatments, the possible effects of random noise or alternating current stimulation, and the combined effects of multiple stimulation targets. Concerning multiple targets, a fascinating new direction of research opened by Grimaldi et al (2014) in patients with cerebellar disorders is multi-target DC stimulation: DC could be used to stimulate the cerebellum, spinal cord and cerebral cortex simultaneously, thus possibly enhancing the induced effects or eliciting still unexplored neuromodulatory responses. Finally, regardless of the stimulation site, research needs to assess whether the electric field modulates inflammatory, reparative or regenerative processes within the CNS. If so, DC electric fields could in theory be used to influence basic disease mechanisms: for instance to modulate inflammatory processes, promote healing and help in regenerating neural elements after CNS injuries (Rueger *et al.*, 2012; Haan & Song, 2013).

Although the cerebellum and spinal cord can be stimulated non-invasively with repetitive magnetic stimulation (Ugawa *et al.*, 1995; Knikou, 2013), neither technique has raised widespread interest for neuromodulation. Cerebral tDCS has several practical advantages over repetitive magnetic stimulation (Priori *et al.*, 2009). These also apply to DC stimulation

of the cerebellum and spinal cord: simplicity, low-cost, portability and wearability. Hence, because these two novel neuromodulation techniques are simple to apply, they promise to be increasingly used in the near future.

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Table 1: Studies (listed in chronological order) using transcranial cerebellar direct current stimulation

Authors	Year	Subjects	Patients	Polarity	Montage	Parameters	Technique	Effects

(cerebellar tDCS)

Non Motor Functions								
Ferrucci et al.	2008	13	\	Anodal\Cathodal\Sham	Active electrode over the cerebellum Reference on the right shoulder	2 mA; 15 min; AEA = 35 cm^2	Sternberg Task	A and C cerebellar tDCS both impaired the practice-dependent improvement in the reaction times
Boehringer et al.	2012	40	\	Cathodal\Sham	Active electrode over the right cerebellum Reference on the buccinator muscle	2 mA; 25 min; AEA =25 cm^2	Forward and Backward Digit Spans	C-cerebellar tDCS reduced forward digit spans and blocked the practice-dependent increase in backward digit spans
Pope and Miall	2012	22	\	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the right shoulder	2 mA; 20 min; AEA =25 cm^2	Paced Auditory Serial Subtraction Task and Paced Auditory Serial Addition Task	Right C-cerebellar tDCS affects working memory and attention differentially depending on task difficulty
Ferrucci et al.	2013	21	\	Anodal\Sham	Active electrode over the cerebellum Reference on the right shoulder	2 mA; 20 min; AEA = 35 cm^2	Serial Reaction Time Task	A-cerebellar tDCS improved procedural learning
Ferrucci et al.	2013	21	\	Anodal\Cathodal\Sham	Active electrode over the cerebellum Reference on the right shoulder	2 mA; 20 min; AEA= 35 cm^2	Facial Emotion Recognition Task	A and C cerebellar tDCS significantly enhanced the response to negative facial emotions
Macher et al.	2014	16	\	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the right buccinator muscle	2 mA; 25 min; AEA =25 cm^2	Sternberg Task	A-cerebellar tDCS causes an attenuated memory recognition capacity and hemodynamic signals
Motor Functions								
Galea et al.	2009	16	\	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the ipsilateral buccinator muscle	2 mA; 25 min; AEA = 25 cm^2	Cerebello-Brain Inhibition	tDCS can modulate in a focal and polarity-specific manner the cerebellar control of the brain
Jayaram et al.	2012	17	\	Anodal\Cathodal\Sham	Active electrode over the right/left cerebellum Reference on the ipsilateral buccinator muscle	2 mA; 15 min; AEA=25 cm^2	Split-belt Walking Task	A-cerebellar tDCS applied during walking improved locomotor adaptation, whereas C-cerebellar tDCS worsened it
Galea et al.	2012	72	\	Anodal\Sham	Active electrode over the right cerebellum Reference on the ipsilateral buccinator muscle	2 mA; 15min; AEA = 25 cm^2	Visuomotor Adaptation Paradigm	Cerebellar tDCS caused faster adaptation to the visuomotor transformation
Hamada et al.	2012	18	\	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the ipsilateral buccinator muscle	2 mA; 15min; AEA=25 cm^2	Paired Associative Stimulation	Plasticity induced by PAS25 was blocked by concurrent A- and C cerebellar tDCS
Sadnika et al.	2013	12	\	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the ipsilateral buccinator muscle	2 mA; 20 min; AEA=25 cm^2	Motor Surround Inhibition	Neither A or C cerebellar tDCS modulated the magnitude of mS
Shah et al.	2013	8	\	Anodal\Cathodal\Sham	Active electrode over the non dominant cerebellum Reference over the ipsilateral buccinator muscle	1 mA; 15 min; AEA = 8 cm^2	Ankle Visuomotor Learning	A and C cerebellar tDCS improved target-tracking accuracy of the ankle
Grimaldi & Manto	2013	\	9	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the contralateral supra-orbital area	1 mA; 20 min; AEA = 20 cm^2	Stretch Reflex Responses in upper limb	A-cerebellar tDCS reduced the amplitudes of long-latency stretch reflexes
Chen et al.	2014	10	\	Anodal\Cathodal\Sham	Active electrode over the right cerebellum Reference on the right buccinator muscle	2 mA; 25 min; AEA = 25 cm^2	Mismatch negativity	A-cerebellar tDCS increased peak amplitude of somatosensory MMN and C-cerebellar tDCS reduced it

Dutta et al.	2014	12	\	Anodal\Sham	Active electrode over the left cerebellum Reference on the forehead above the right supraorbital ridge.	1 mA; 15 min; AEA = 35 cm^2	Voluntary visually-cued muscle activity in the tibialis anterior muscle	A-cerebellar tDCS worsened different aspects of visually-cued voluntary contraction of a lower limb muscle
Grimaldi et al.	2014		2	Anodal\Sham	Active electrode over the right cerebellum followed by contralateral motor cortex Reference on the contralateral supra-orbital area	1 mA; 20 min; AEA = 20 cm^2	Upper limb tremor and dysmetria	Cerebello-Cerebral tDCS improves upper limb tremor and hypermetria
Hardwick & Celnik	2014	33		Anodal\Sham	Active electrode over the lateral cerebellum (dominant hand) Reference on the Ipsilateral buccinators muscle	2 mA; 15 min; AEA = 25 cm^2	Motor adaptation	A-cerebellar tDCS enhances motor adaptation in older individuals
Zuchowski et al.	2014	30		Anodal\Sham\Cathodal	Active electrode over the right cerebellum Reference on the right buccinator muscle	2 mA;during the acquisition phase AEA = 35 cm^2	Eyeblink conditioning	A-cerebellar tDCS enhances acquisition conditioned eyeblink responses; C-cerebellar tDCS reduced it
Herzfeld et al.	2014	50		Anodal\Sham\Cathodal	Active electrode over the right cerebellum Reference on the right buccinator muscle	2 mA; 25 min; AEA = 25 cm^2	Force field learning	A-cerebellar tDCS enhances the error-dependent learning process; C-cerebellar tDCS impaired it

A-cerebellar tDCS: anodal cerebellar tDCS; C-cerebellar tDCS: cathodal cerebellar tDCS; mA: milliampere; AEA: active electrode area

Table 2: Studies (listed in chronological order and according to the evaluated physiological system) using transcutaneous spinal direct current stimulation (spinal tDCS) studies in humans (top) and animals (bottom).

STUDIES IN HUMANS								
Authors	**Year**	**Subjects**	**Patients**	**Polarity**	**Montage**	**Parameters**	**Technique**	**Effects**
Ascending Pathways								
Cogiamanian et al.	2008	12	\	Anodal\Cathodal	Active electrode at Th11 level Reference on right shoulder	2.5 mA; 15 min; AEA = 35 cm^2	Somatosensory Evoked Potentials	Changes in conduction along human lemniscal pathway
Truini et al.	2011	20	\	Anodal\Cathodal	Active electrode at Th11 level Reference on right shoulder	2.5 mA; 20 min; a AEA = 35 cm^2	Laser Evoked Potentials	A-spinal tDCS might impair conduction in the ascending nociceptive spinal pathways, thus modulating LEPs and increasing pain tolerance.
Descending Pathway								
Lim and Shin	2011	12	\	Anodal\Cathodal\Sham	Active electrode at C7 level Reference on the anterior neck	2 mA; 20 min; AEA = 25 cm^2	Motor Evoked Potentials	Spinal tDCS can induce an increase in corticospinal tract excitability
Spinal Reflexes								
Winkler et al.	2010	10	\	Anodal\Cathodal\Sham	Active electrode at Th11 level Reference on right shoulder	2.5 mA; 15 min; AEA = 35 cm^2	H-Reflex Post-Activation Depression	A-spinal tDCS increases the efficacy of the Ia fibre-motorneurone synapse, whereas c-tsDCS reduces it
Cogiamanian et al.	2011	11	\	Anodal\Sham	Active electrode at Th11 level Reference on right shoulder	2 mA; 15min; AEA= 35 cm^2	Lower Limb Flexor Reflex	A-spinal tDCS elicited long-lasting after-effects on central nociceptive signal transmission
Lamy et al.	2012	17	\	Anodal\Cathodal\Sham	Active electrode at Th11 level Reference on right shoulder	2.5 mA; 15 min; AEA = 35 cm^2	H Reflex recruitment Curve	Spinal tDCS is capable of inducing enduring changes in spinal segmental excitability that last for at least 15 min after current offset
Lamy and Boakye	2013	17	\	Anodal	Active electrode at Th11 level Reference on right shoulder	2.5 mA; 15 min; AEA = 35 cm^2	H Reflex recruitment Curve	BDNF Val66Met genotype impacts spinal plasticity in humans, as assessed by a-spinal tDCS
Hubli et al.	2013	17	17	Anodal\Cathodal\Sham	Active electrode at Th11 level Reference on right shoulder	2.5 mA; 20 min; AEA = 35 cm^2	Spinal Reflex Behaviour	A-spinal tDCS and assisted locomotion induce changes in SR behavior in SCI subjects; C-spinal tDCS and sham lead to a drop in SR amplitudes in healthy subjects
EXPERIMENTS ON ANIMALS								
Authors	**Year**	**Sample/ Rat**	**Patients**	**Polarity**	**Configuration**	**Parameters**	**Technique**	**Effects**
Aguilar et al.	2011	44	\	Anodal\Cathodal	Active electrode on thoracic spinal cord Reference on anterior abdominal area	1 mA; 15 min; AEA = 0.79 cm^2	Somatosensory Evoked Potentials	A-spinal tDCS increases spontaneous activity in the gracile nucleus while decreasing its local field potentials responses to somatosensory stimuli; C-spinal tDCS did the opposite
Spinal Network								
Ahmed	2011	33	\	Anodal\Cathodal	Active electrode at T10-L1 Reference on lateral abdominal muscles	from 0.5 mA to 3 mA for 3 min; AEA = 0.79 cm^2	Spontaneous activity recording and rCES	A-spinal tDCS increases the spike frequency and the amplitude of spontaneous discharges; c-spinal tDCS + rCES increases cortical elicited twitches

Ahmed and Wieraszko	2012	87	Contusive and hemisectione mice	Anodal\Cathodal	Active electrode at T10-L1 Reference on lateral abdominal muscles	2 mA; 5 secs; AEA = 0.79 cm^2	Cortical elicited muscles action and in vitro glutamate	Combination of C-spinal tDCS/rCES enhances spinal cord responses in control and SCI animals; C-spinal tDCS/rSS releases the maximum amount of glutamate
Ahmed	2013	116	Unilateral SCI	Cathodal	Active electrode at T13-L4 (spinal level L3-L6) Reference on abdominal skin flap	0.8 mA; different durations; AEA = 3.5 cm^2	Associative Plasticity	C-spinal tDCS + SSA or CSA is able to increase associative plasticity in healthy animals and to improve recovery from unilateral SCI.
Ahmed	2013	30	\	Cathodal	Active electrode on lumbar enlargement area Reference on abdominal skin flap	0.8 mA; 8 sec; AEA = 3.5 cm^2	Spinal Network and Complex Multijoint Movements	C-spinal tDCS modulates the kinematic of elicited movements and bursting activity in spinal circuitries, probably through activating the spinal GABAergic system

Th11: eleventh thoracic vertebrae; C7: seventh cervical vertebrae; BDNF: brain derived neurotrophic factor; Val66Met: valine-to-methionine substitution at codon 66; SCI: spinal cord injury; SR: spinal Reflex; rCES: repetitive cortical electrical stimulation; rSS: repetitive spinal stimulation; SSA: spino-sciatic associative stimulation; CSA: cortico-sciatic associative stimulation; mA: milliampere; A-spinal tDCS: anodal spinal tDCs; C-spinal tDCS: cathodal spinal tDCS; AEA: active electrode area.

Figure 1: Effects of cerebellar transcranial direct current stimulation (cerebellar tDCS) on tracking accuracy as a function of time across different stimulation conditions. The y-axis depicts the accuracy index (AI) normalized to baseline during practice, and the x-axis shows the different time points: baseline, 10 min after practice (Post 10), 30 min after practice (Post 30) and 60 min after practice (Post 60). The normalized accuracy index (nAI) improved more after cathodal cerebellar tDCS, anodal cerebellar tDCS and anodal M1 than after sham and cathodal M1 stimulations at all post time points. From Shah et al. (2013) with permission.

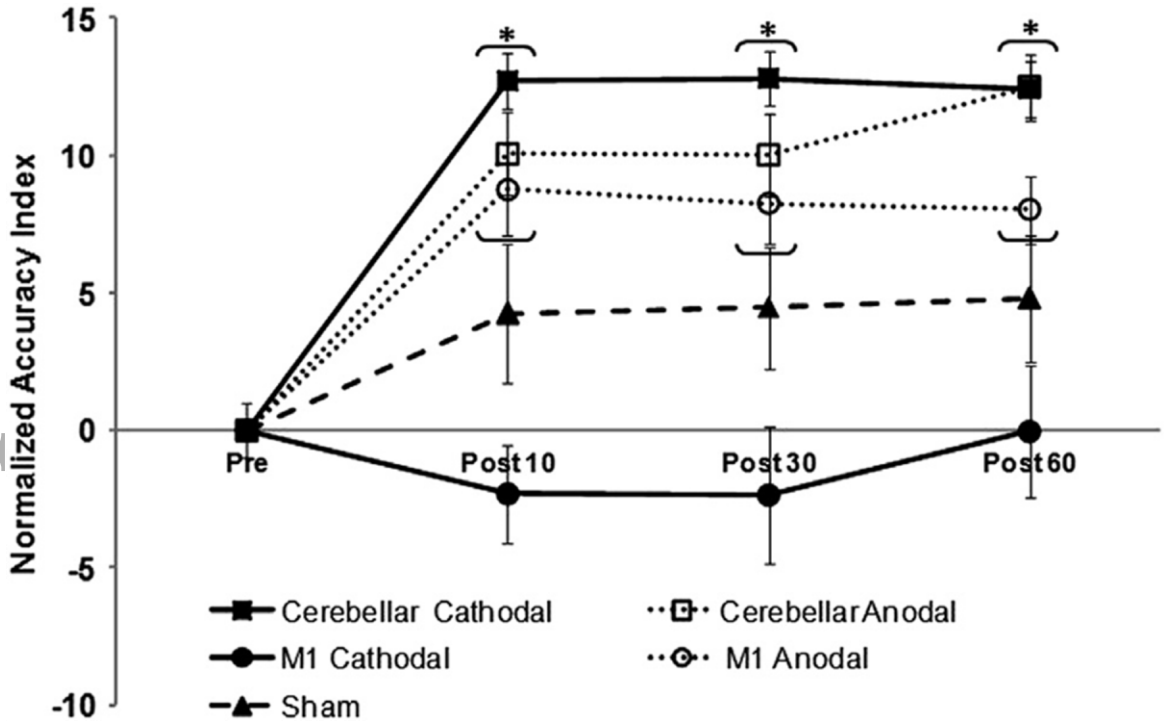


Figure 2: Effect of cerebellar transcranial direct current stimulation (cerebellar tDCS) on stretch reflexes in the upper limb of an ataxic subject. The figure shows rectified and averaged EMG signal from the flexor carpi radialis muscle in a representative cerebellar patient. Reflexes were elicited by passive wrist extension elicited by a torque motor. Anodal cerebellar tDCS left short-latency stretch reflex (SLSR) amplitudes unchanged but reduced amplitudes for long-latency stretch reflexes (LLSR). From Grimaldi and Manto (2013) with permission.

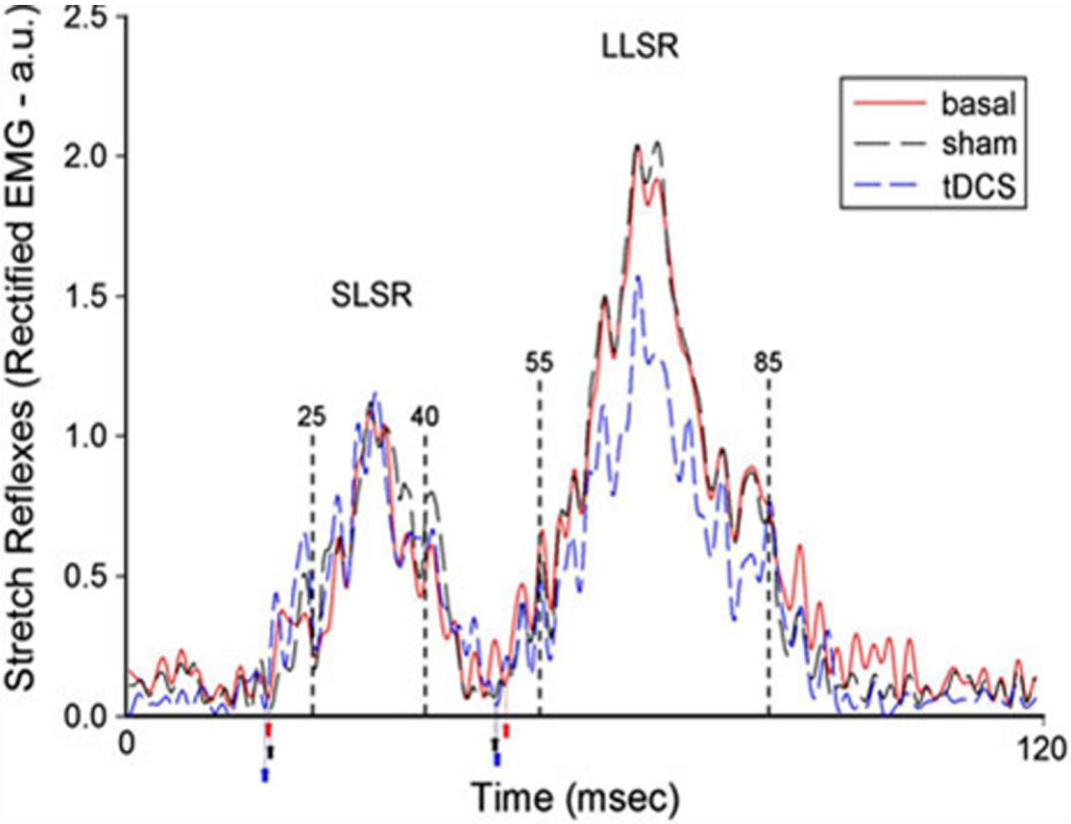


Figure 3. Modelling study on the current density generated by cerebellar transcranial direct current stimulation (cerebellar tDCS) and by transcutaneous spinal cord direct current stimulation (spinal tDCS) in humans. Top panels show a schematic drawing illustrating the electrode positions for cerebellar tDCS (A) and spinal tDCS (B). C: segmentation masks for three human realistic Virtual Family models undergoing cerebellar tDCS. (a) Lateral view of grey matter, cerebellum, pons, midbrain, medulla; (b) Lateral view of the skull; (c) Back view of the cerebellum. (d) and (e) Lateral and inferior views of current density amplitude field distributions over cortical, subcortical and brain-stem regions across all models; (f) back view of current density amplitude field distributions over the cerebellum. Note that cerebellar tDCS generates the highest current density in the posterior cerebellum with a slight spread to other structures. From Parazzini et al (2014a) with permission. D: segmentation masks for three human realistic Virtual Family models undergoing spinal tDCS. (a) Lateral view of skull, vertebrae, intervertebral disks, cerebrospinal fluid and nerves; (b) Magnified clipped frontal view of the spine around the tenth thoracic vertebra, showing vertebrae, intervertebral disks, spinal cord, cerebrospinal fluid and nerves; (c) Lateral and frontal (d) views of the current density amplitude field distributions over spinal cord and nerves. Note that when the reference electrode is over the right arm the acts mainly at thoracic level with minimal current spread. From Parazzini et al (2014b) with permission.

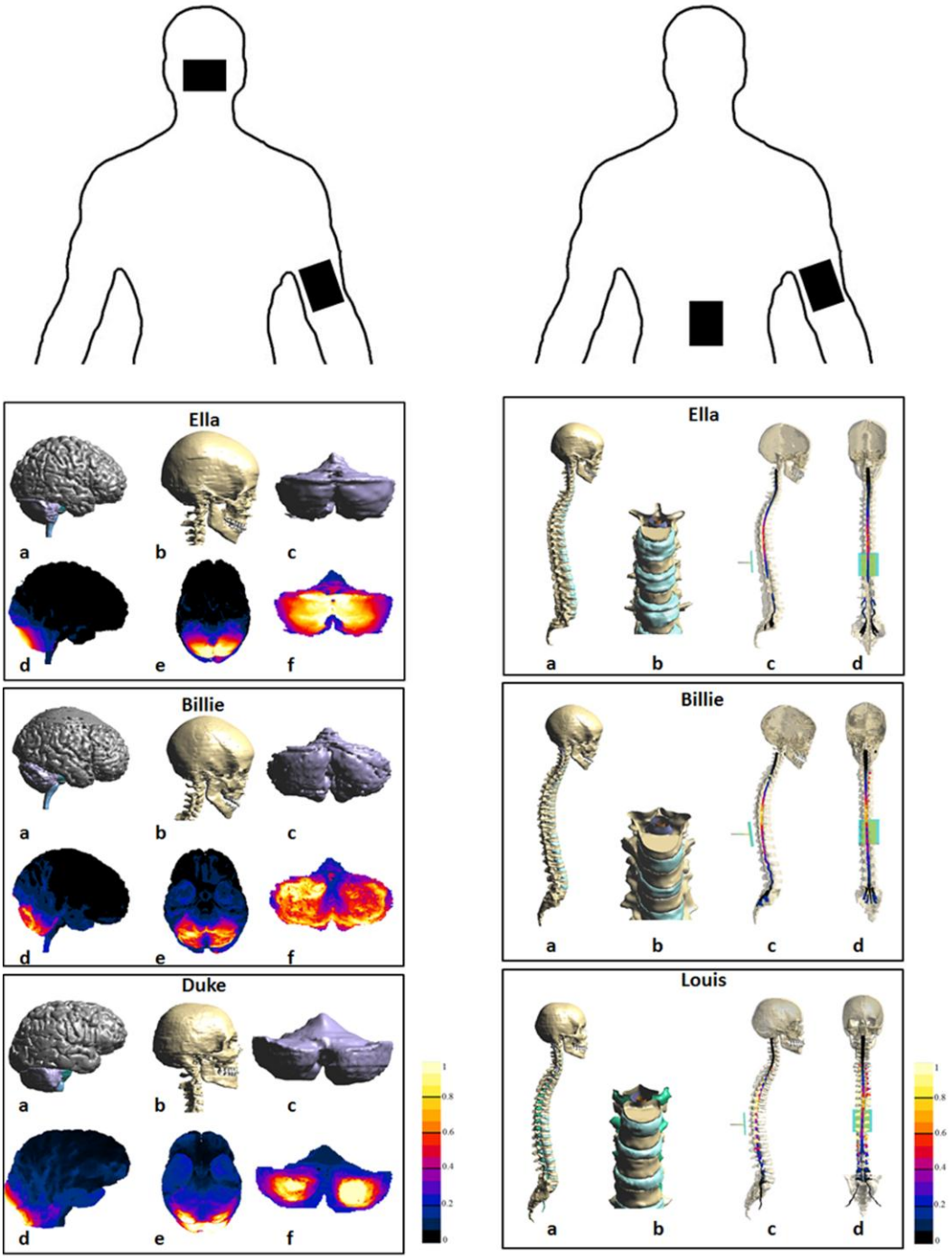


Figure 4: Effects of transcutaneous spinal cord stimulation (spinal tDCS) on the somatosensory evoked potentials (SEPs). SEPs were obtained after posterior tibial nerve stimulation at the ankles. Activity was measured at the popliteal fossa (popliteal potential, N9), at the first lumbar vertebra referred to the umbilicus (spinal potential, N22), at the sixth cervical vertebra referred to Fpz (cervico-medullary potential, P30) and at Cz referred to the right earlobe (cortical potential, P39). The amplitude and latency for each SEP component were measured at two time points (baseline = before spinal tDCS, T20 = 20 minutes after spinal tDCS offset). From the top, pairs of traces are P39, P30, N22 and N9. In each pair, the top trace is the baseline recording (B), whereas the bottom trace is recorded 20 minutes after DC offset (T20). Note that anodal spinal tDCS (2.5 mA. 15 minutes) on the eleventh thoracic vertebra modulates the cervico-medullary potential (P30, second pair of traces, grey lines), inducing a long-lasting effect, 20 minutes. Conversely spinal tDCS leaves the other potentials unchanged. Figure modified from Cogiamanian et al., 2008, with permission.

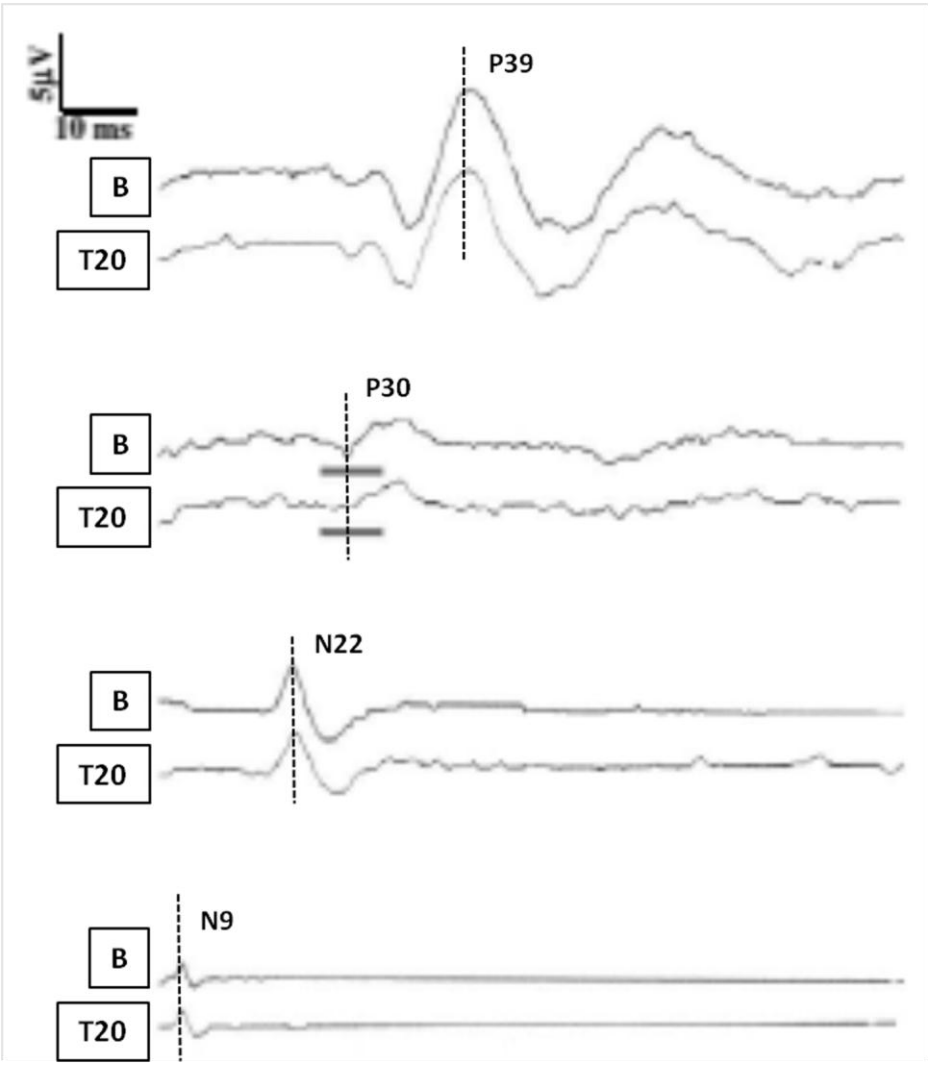


Figure 5: Effects of transcutaneous spinal cord direct current stimulation (spinal tDCS) on laser evoked potentials (LEPs). In A, an example showing the spinal tDCS set up: active electrode on the lower thoracic spinal cord, reference electrode on the right shoulder. In B, perioral and foot LEPs in a representative subject. Laser pulses were applied to the right perioral region (Panel B1) and to the right foot (Panel B2). LEP N2 and P2 components were recorded from the vertex (Cz with reference on the nose); the N1 component was recorded from the temporal area (T3, reference on Fz). Peak latency and baseline to peak amplitude were measured before and after anodal (on the left) and cathodal (on the right) stimulation. In B1, perioral LEPs before (top row) and after (bottom row) anodal and cathodal DC (2.5 mA, 20 minutes). In B2, foot LEPs before (top) and after (bottom) spinal tDCS. Anodal spinal tDCS decreased N1 and N2 amplitude of foot-evoked LEPs but not of perioral evoked responses (B2, grey circle). Figure modified from Truini et al., 2011, with permission.

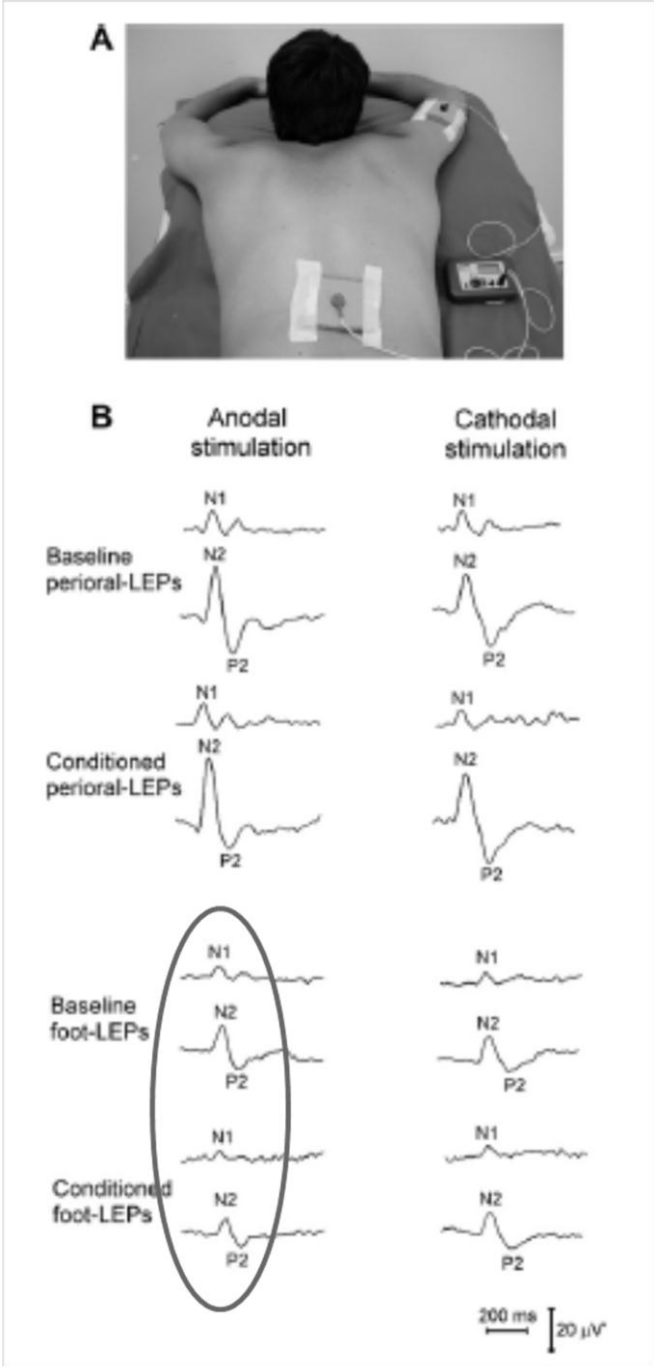


Figure 6: effects of anodal transcutaneous spinal cord direct current stimulation (spinal tDCS) on the lower limb flexion reflexes (LL-Fr) (A). The trace B (baseline) shows a typical LL-Fr recording from a healthy subject. LL-Fr is a polysynaptic spinal reflex elicited by electrical stimulation applied to a sensory nerve. LL-Fr comprises an early response (RIIr) and a late response (RIIIr). RIIIr is a high-threshold nociceptive A-delta fibre mediated reflex that corresponds to the pain threshold (RIIIr threshold) and pain perception (RIIIr size). LL-Fr was elicited from the sural nerve and responses were recorded from the ipsilateral brevis head of the biceps femoris muscle. The stimulus (5 electrical pulses, pulse duration 1 ms, frequency 200 Hz) was delivered randomly every 5–20 seconds. The stimulus intensity was set at 120% of RIIIr threshold (average of 5 responses for each leg). RIIIr decresed after anodal spinal tDCS (A), (grey circle) immediately after (T0) and 30 minutes (T30) after stimulation ended. Sham stimulation left RIII area unchanged (Panel B). Figure modified from Cogiamanian et al., 2011, with permission.

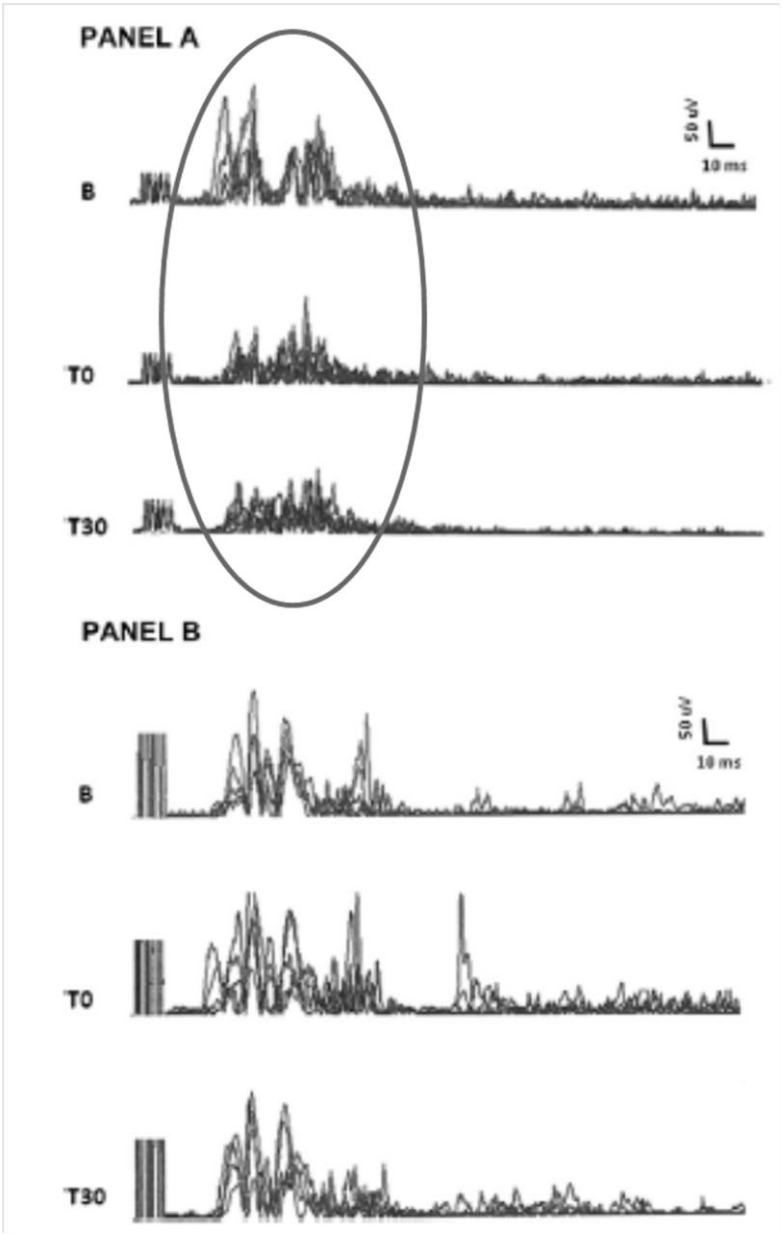
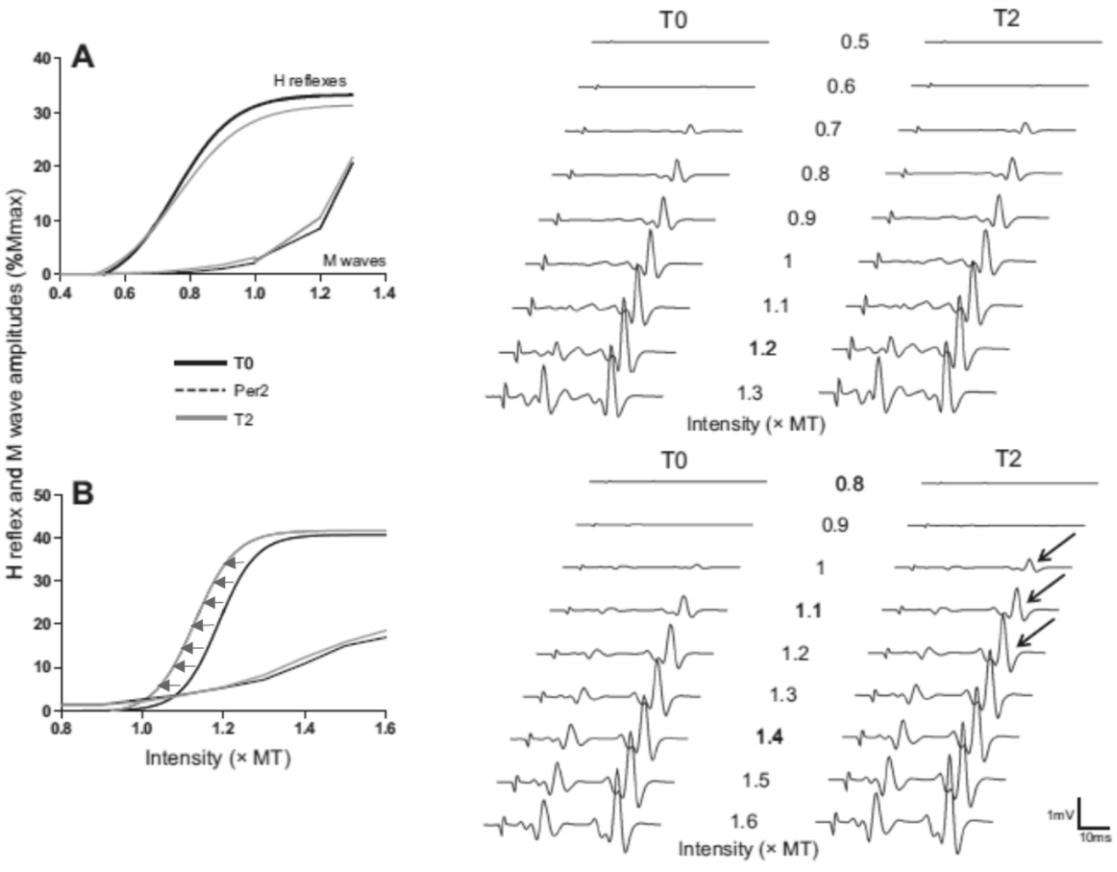


Figure 7: Effects of transcutaneous spinal cord direct current stimulation (spinal tDCS) on the H-reflex recruitment curve. The soleus H reflex was elicited by stimulating the posterior tibial nerve with pulses lasting 1-ms at a stimulation frequency of 0.33 Hz. The stimulation **i**ntensity was progressively increased in 5/10% steps of the threshold intensity to evoke an M wave. Five responses were averaged at each stimulus intensity. On the left, the figure shows the differential effect of spinal tDCS on a methionine allele carrier (A) and valine/valine carrier (B). Note that anodal spinal tDCS shift to the left the curve only in subjects with the valine/valine polymorphism in the BDNF gene (B, leftward shift in the H-reflex recruitment curve, red arrows). On the right side, graphic representation showing the effect of anodal spinal tDCS in two representative subjects, one from each group (Top traces: methionine allele carrier; bottom traces: valine carrier). Black arrows indicate amplitude changes after current offset (T2).. Note that in the methionine carrier the H-reflex appeared at the same stimulation intensity (top), whereas in the valine carrier the H-reflex appeared at lower intensity after spinal tDCS. T0: baseline; Per2: second online recording; T2: 15 minutes after DC stimulation offset; MT: threshold intensity to evoke an M wave. Figure adapted from Lamy et al., 2012, with permission.



Additional information

Competing Interests

Alberto Priori, Maurizio Vergari and Roberta Ferrucci are stakeholders in Newronika s.r.l., a spin-off company formed by the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Università degli Studi di Milano.

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